Spec2Seq Manual

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Introduction

Spec2Seq offers the opportunity to translate precision polymer fragment spectra into their corresponding monomer sequences. It is especially designed for the analysis of precision polymer libraries, but can also be adapted to different systems by providing a tool for creating a personal monomer block library. Furthermore, it can be used to assign peaks directly from known sequences by using the in-built fragment predictor tool. In the next few pages the P*recision Polymer DeNovo Sequencing*, *Monomer Block Library* and *Fragment Predictor* Tool will be explained.

Precision Polymer DeNovo Sequencing

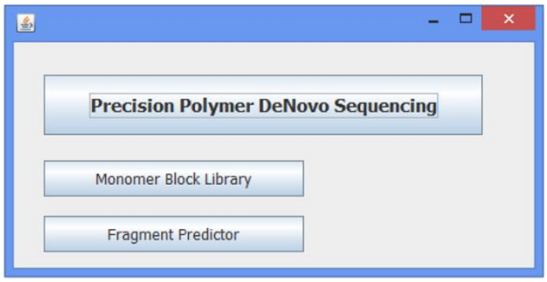

Figure 1. Start the software by double clicking Spec2Seq.jar. The start-screen of Spec2Seq opens, providing the three options *Precision Polymer DeNovo Sequencing*, *Monomer Block Library* and *Fragment Predictor*. Choose *Precision Polymer DeNovo Sequencing*.

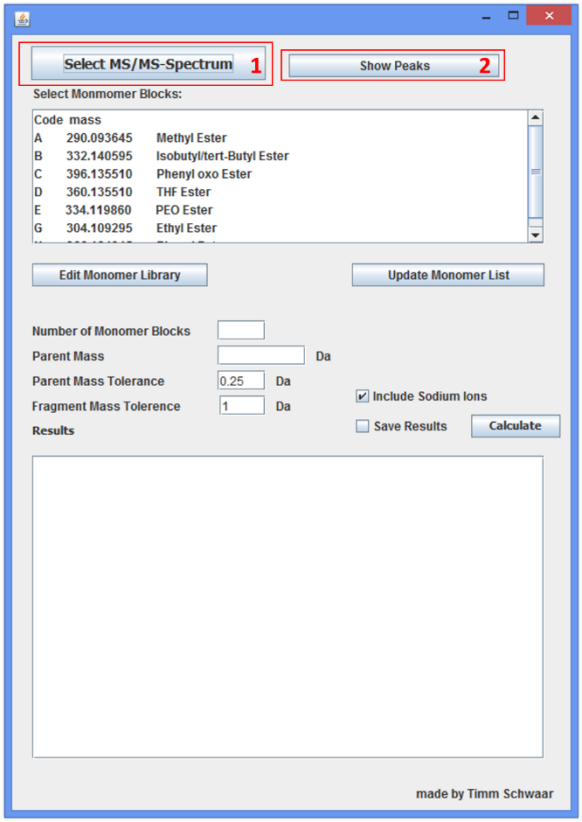

Figure 2. A new interface opens, showing several buttons and options. Start by selecting your ASCII file (e.g. *.txt, *.dat) fragment spectrum file (*Select MS/MS-Spectrum*) and load it into the software (**1**). Click on *Show Peaks* to see all peaks considered for analysis (**2**). The precision polymer A-B-C-G-G was chosen for demonstration.

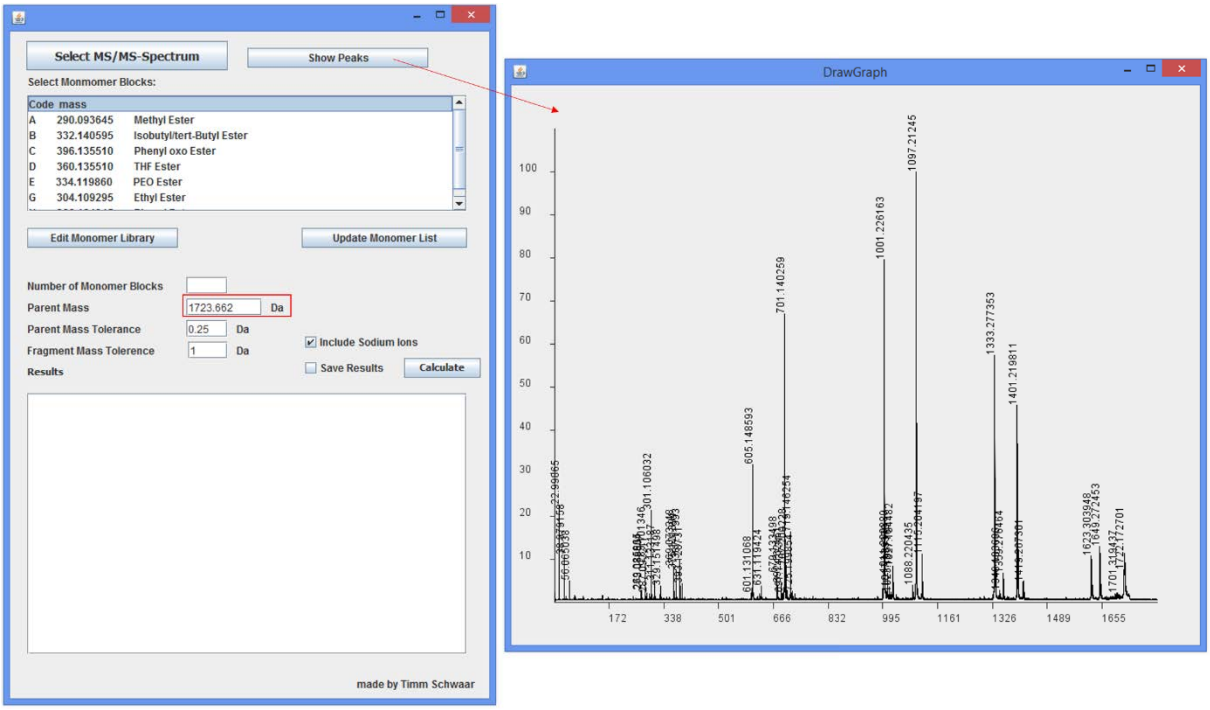

Figure 3. After clicking on *Show Peaks* a second window opens, showing the spectrum and considered peaks for analysis. Note that depending on your ASCII file the *Parent Mass* is already filled with the correct value. If not, please insert manually.

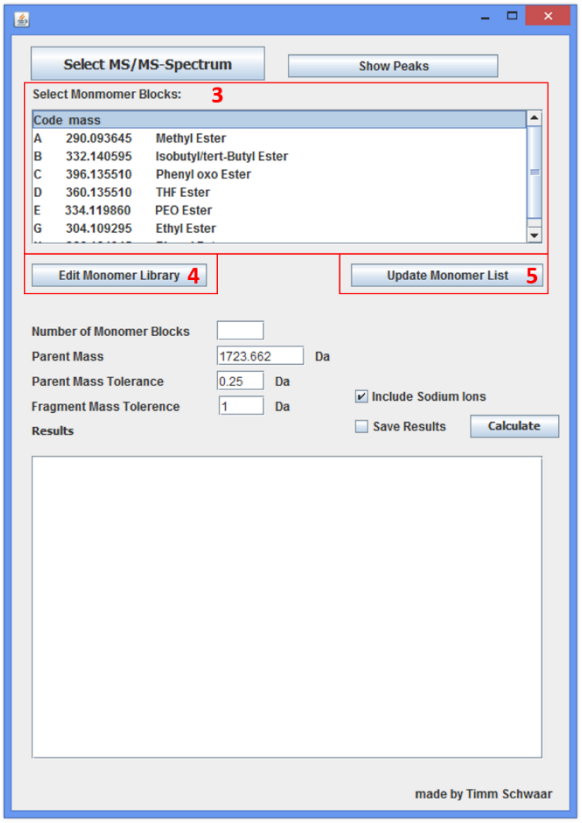

Figure 4. The monomer blocks used in the precision polymer library can now be easily selected by double-clicking either on the name, the code or the mass of the monomer (**3**). If your monomer is not yet in the library it can manually be added by clicking *Edit Monomer Library* (**4**). The procedure will be explained in section *Monomer Block Library*. After addition of the newly added monomer block, update the software by clicking *Update Monomer List* (**5**).

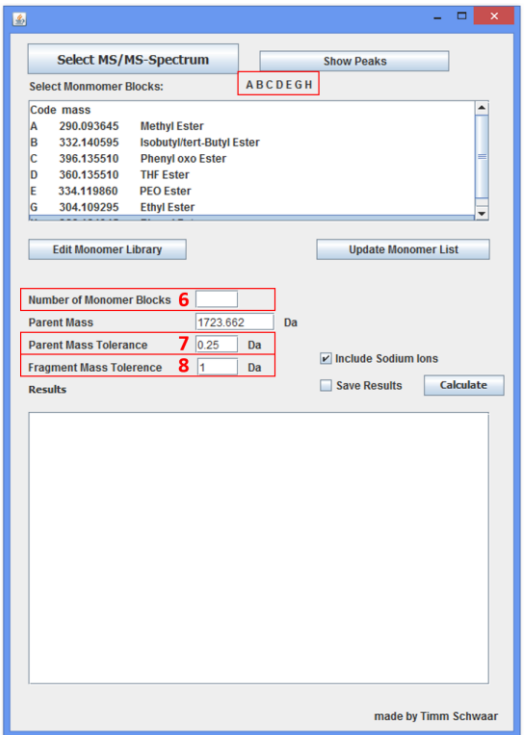

Figure 5. The selected monomer blocks now appear next to *Select Monomer Blocks* and can be removed by double-clicking the name of the block in the list. The number of monomer blocks has to be inserted manually (**6**). Default values are already inserted for *Parent Mass Tolerance* and *Fragment Mass Tolerance* and are recommended to use. However, also these values can be edited (**7**) and (**8**).

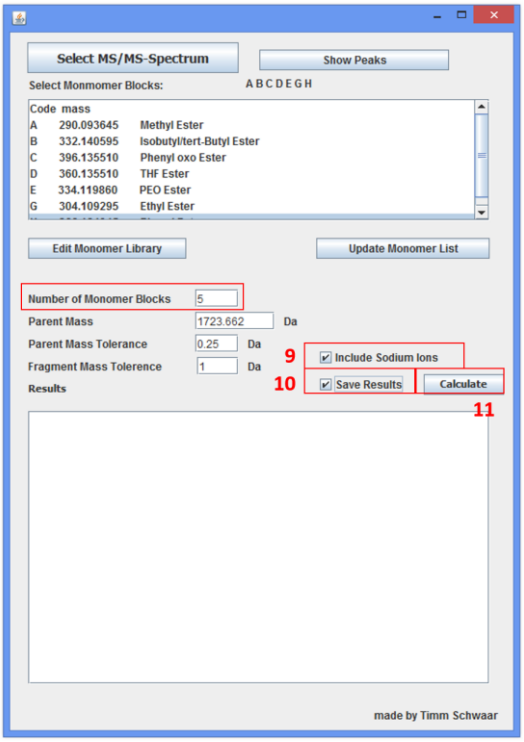

Figure 6. Note that the number of monomer blocks is now filled. For some measurements it is necessary to include sodium ions (**9**), choose only if necessary. The results of the calculations can be saved as *.txt (**10**). Click *Calculate* to start the analysis (**11**).

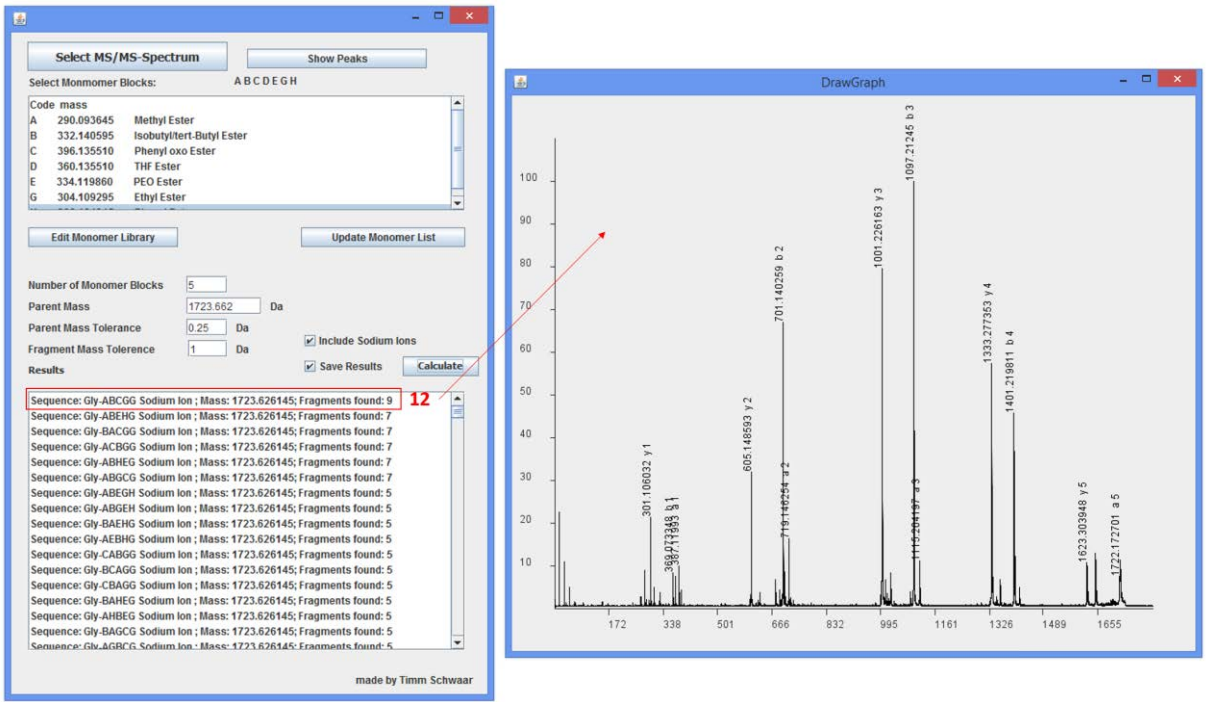

Figure 7. The output of the calculation appear in the result window. They are sorted by the number of fragments found, with the highest number on top. Note that only b- and y-fragments count to the final number. However, the a- and x-fragments are shown in the result spectrum, showing the assigned peaks. It can be opened by double-clicking the name of the sequence (**12**).

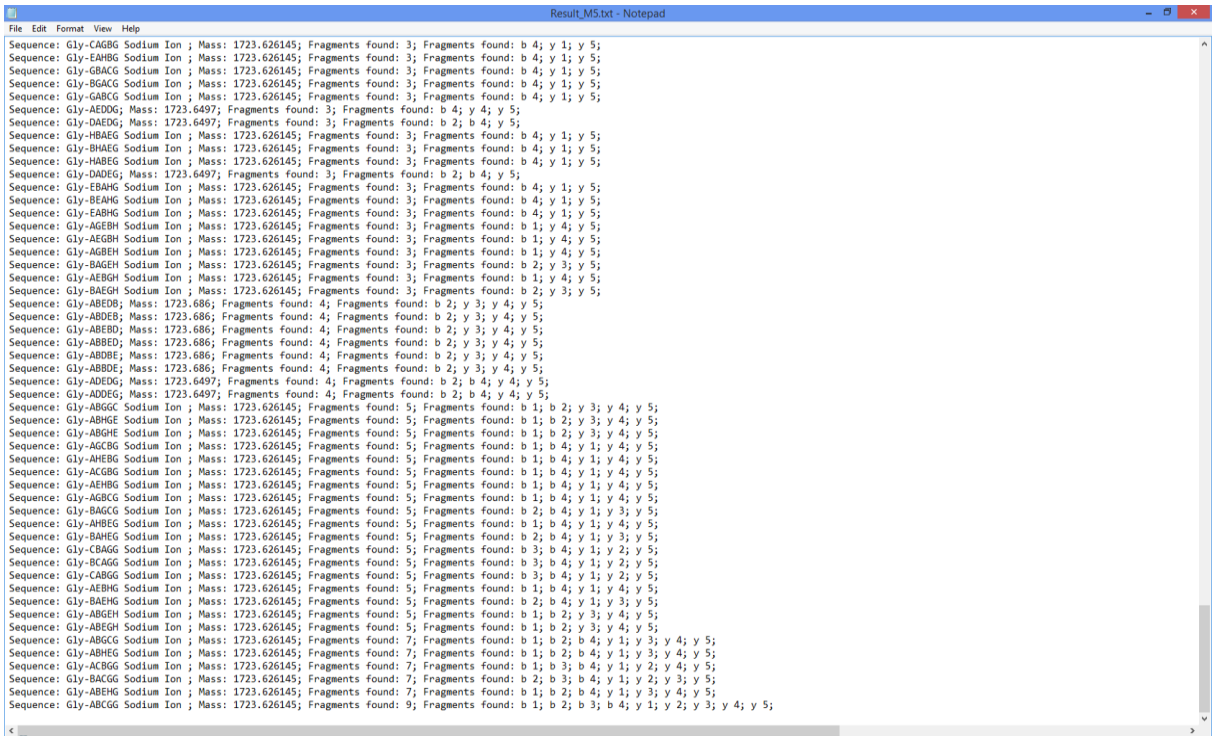

Figure 8. For detailed description such as which fragments were found the result.txt file can be opened.

Monomer Block Library

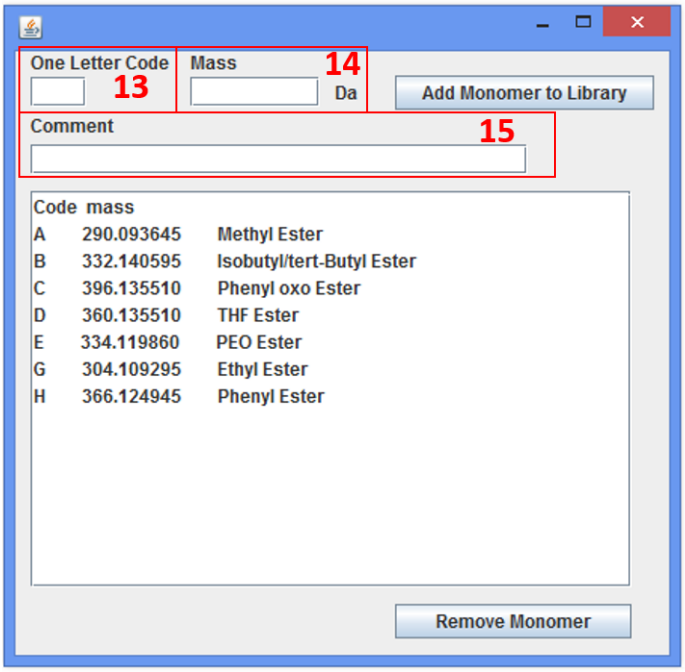

Figure 9. For adding a new monomer to the library a one letter code has to be created (**13**). Note that also numbers are allowed as one letter codes. Next, enter the exact mass of the building block as diradical (**14**). An additional comment can be added such as the full monomer name (**15**). Click *Add Monomer to Library*.

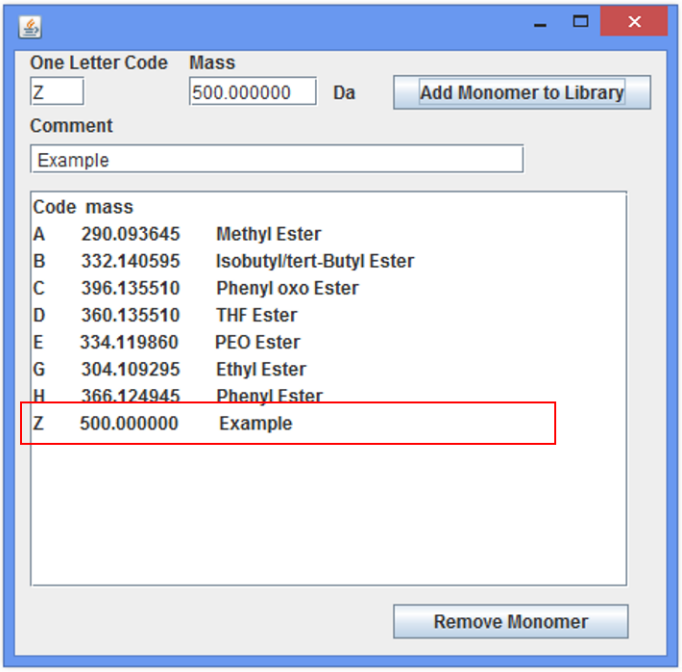

Figure 10. A monomer block was added with the one letter code “Z”, the mass “500.000000” and the comment “Example”. It appears now in the library.

Fragment Predictor

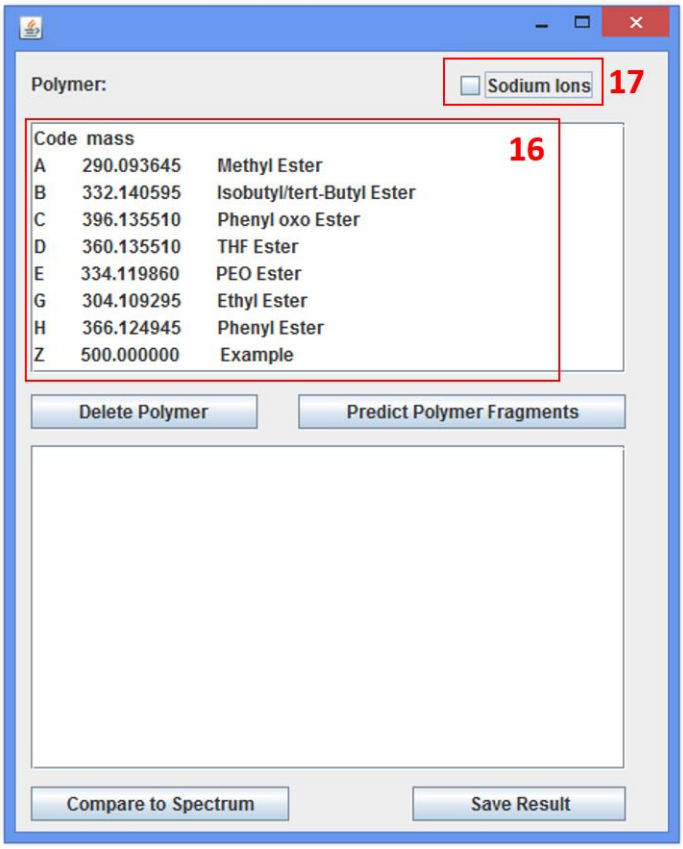

Figure 11. The fragment predictor can be used building up the precision polymer by double clicking the monomers (**16**). It can be chosen to include sodium ion (**17**). By clicking *Predict Polymer Fragments* the software calculates the expected fragments.

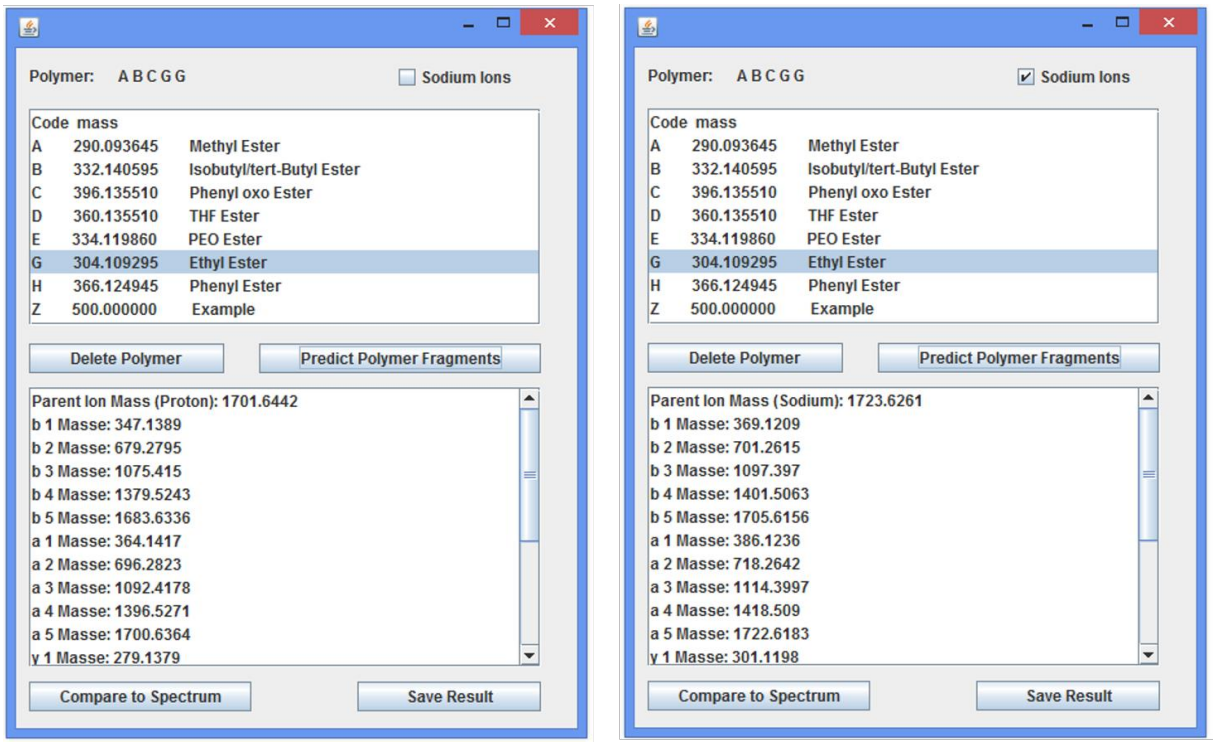

Figure 121. Results of the calculations excluding and including sodium ions.

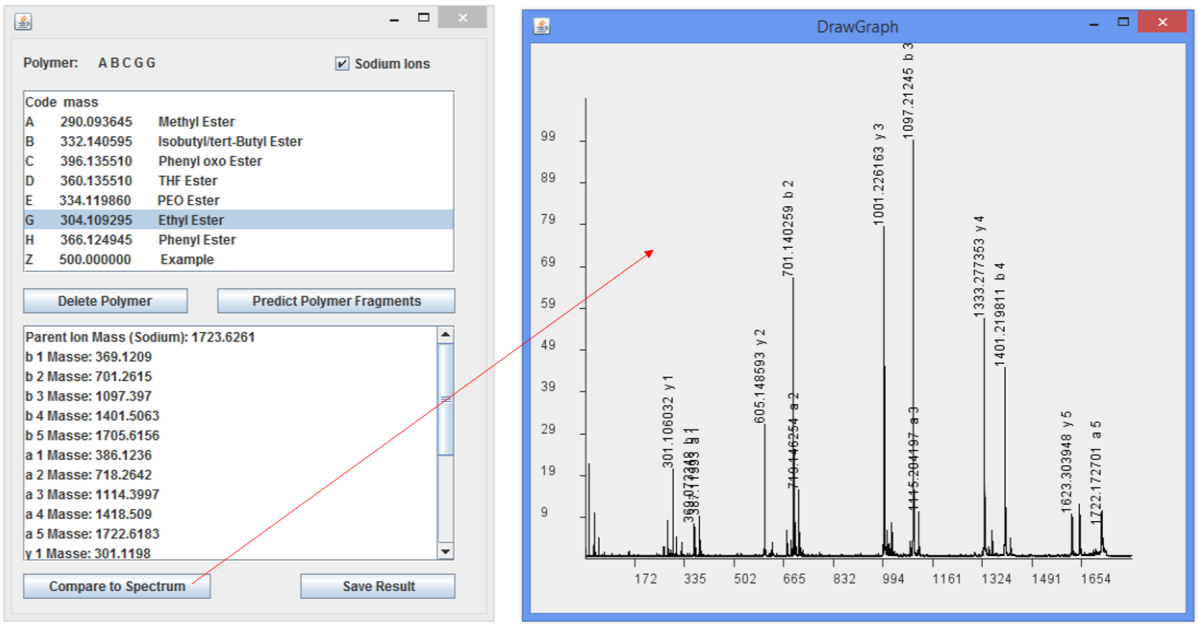

Figure 13. The button *Compare to spectrum* offers the direct comparison to a measured spectrum. This tool comes in handy for known sequences.

FAQ

1. The result shows “no match”.

   A: This error appears when no sequences can be assigned. Often the parent mass is wrong/missing, or the number of monomer blocks is wrong. Also check whether the *Sodium Ions* button is selected.

2. Length is empty.

   A: Enter a value for the building blocks and try again.

3. No spectrum selected.

   A: The selected spectrum has the wrong format or cannot be read. Only ASCII files (e.g. *.txt, *.dat) accepted.